PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application

Ligensa, et al.

Group: 1646

Examiner: J. Andres

Serial No. 09/453,195, filed December 2, 1999

For: IGF-1 RECEPTOR INTERACTING PROTEINS

DECLARATION OF MICHAEL WEIDNER UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Michael Weidner, a citizen of Germany, declare as follows, that:

In 1991 I received a Ph. D in Cell Biology summa cum laude from the University of Essen, Essen, Germany.

From 1991 through 1993 I did postdoctoral research at the Institute of Cell Biology at the University of Essen, Essen Germany and the Institute of Cell Biology at the Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, and received the "Falcon Preis" awarded by theGerman Society for Cell Biology for work done during this period.

Since November 1996 I have been employed by Hoffmann-La Roche Ltd. as Senior Scientist in the Department of Oncology and Department of Molecular Biology, Penzberg, Germany. My research is in the area of molecular mechanisms of tumor development and progression, with regard to signal transduction, growth factors, tyrosine kinases, and adhesion molecules, using recombinant cell systems, gene discovery, protein biochemistry, and yeast two-hybrid technology.

I am an inventor of the claimed nucleic acids which encode the protein IIP-10 which binds to the IGF-1 receptor. This Declaration is submitted to demonstrate that IIP-10 binds to and inhibits the activity of the IGF-1 receptor.

To make this demonstration, the following experiments of Examples 1 and 2 were carried out under my supervision and control.

Examples

Example 1:

The IGF-1 receptor stimulates tumor cell proliferation. IIP-10 expression inhibits this proliferation in IGF-1 receptor overexpressing NIH-3T3 cells which stably express IIP-10.

Example 2:

The IGF-1 receptor protects tumor cells from Fas-induced apoptosis. IIP-10 expression reduces the level of protection accordingly increasing apoptosis in IGF-1 receptor overexpressing NIH-3T3 cells which stably express IIP-10.

Materials

NIH-3T3 fibroblast clones which overexpress the human IGF-1 receptors and therefore have acquired the characteristics of tumor cells: 10/2, 10a/12, 10a/19, 10a/20, H2, and H3.

Clones stably transfected with IIP-10: 10/2, 10a/12, 10a/19, and 10a/20.

Clones not transfected with IIP-10 (mock transfected clones): H2, H3

Methods

Example 1: IIP-10 inhibition of IGF-1 induced stimulation of proliferation in IGF-1 receptor overexpressing NIH3T3 cells:

Four IGF-1R overexpressing NIH3T3 clones stably transfected with IIP-10 (10/2, 10a/12,

10a/19, 10a/20) and two mock transfected clones (H2, H3) were plated in serum containing medium at a density of 5 x 10^3 cells/well in a 96-well microtiter plate. After 24h the growth medium was removed and replaced with serum free medium (SFM) containing 0.5% dialyzed FCS. After serum starvation for 24h, the cells were stimulated with 50ng/ml IGF-1 in SFM containing 0.5% dialyzed FCS for 48h. For monitoring cell proliferation cells were labeled with BrdU (Roche Molecular Biochemicals) for 24h. After the labeling-period a Cell Proliferation ELISA (Roche Molecular Biochemicals) quantifying the incorporated BrdU was performed according to the manufacturers protocol. In Figure1, the ordinate gives the percent increase in BrdU incorporation after IGF-1 stimulation compared to SFM containing 0.5% dialyzed FCS. As can be seen in Figure 1, H2 and H3 cells showed about 250% and about 375% proliferation, respectively. In contrast, the 10/2, 10a/12, 10a/19, and 10/20 cells showed about 150%, 75%, 100%, and 100% proliferation, respectively.

Example 2: IIP-10 inhibition of IGF-1 mediated protection of fas induced apoptosis in IGF-1 receptor overexpressing NIH3T3 cells:

One IGF-1R overexpressing NIH3T3 clone stably expressing IIP-10 (10/2) and one mock transfected clone (H2) were plated in serum containing medium at a density of 5x10^3 cells/well in a 96-well microtiter plate. After 24h, Fas-mediated apoptosis was induced by the addition of 70ng/ml recombinant human FasL (Alexis, CA, USA) and 1µg/ml Enhancer (Alexis, CA, USA) in serum free medium (SFM) containing 0.5% dialyzed FCS. At the same time IGF-1 was added in a concentration of 10^{-8}M to protect cells from Fas-induced apoptosis. After 16h the amount of apoptotic cells was quantified using the Cell Death Detection ELISA^{PLUS} (Roche Molecular Biochemicals) according to the manufacturers protocol. As can be seen in Figure2, about 50% of 10/2 cells suffered apoptosis. In contrast, only about 25% of H2 cells suffered apoptosis.

Conclusion

These results demonstrate that the protein IIP-10 inhibits the action of the IGF-1 receptor in cells which express the IGF-1 receptor.

Example 1 shows that IIP-10 expression reduces proliferation in tumor cells. IIP-10-transfected tumor cell clones showed from 100% to 300% less proliferation than non IIP-10-transfected tumor cell clones. The IGF-1 receptor, when active, promotes tumor cell proliferation. Therefore the ability of IIP-10 to inhibit the activity of the receptor reduces proliferation.

Example 2 shows that IIP-10 expression increases apoptosis in tumor cells. Fas-mediated apoptosis eliminated twice as many IIP-10-transfected tumor cell clones than non IIP-10-transfected tumor cell clones. The IGF-1 receptor, when active, can protect tumor cells from apoptosis. Therefore the ability of IIP-10 to inhibit the activity of the receptor reduces protection from apoptosis and gives rise to more apoptosis.

These results indicate that inhibition of IGF-1 receptor activity has negative effects on tumor cells, reducing their ability to proliferate and increasing their vulnerability to apoptosis. Therefore, the ability of IIP-10 to inhibit the activity of the IGF-1 receptor indicates that IIP-10 has antitumor activity.

In addition, the more IIP-10 is present in a tumor cell, the less likely is the cell to metastasize, since IIP-10 reduces the ability of tumor cells to proliferate. Therefore the IIP-10 level found in a given tumor cell is related to its metastatic potential.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 23.04.01

Michael Weidner

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Publications:

Keilhack H., M Müller, SA Böhmer, C Frank, KM Weidner, W Birchmeier, T Ligensa, A Berndt, H.Kosmehl, B. Günther, T. Müller, C. Birchmeier, FD. Böhmer. Negative Regulation of Ros Receptor Tyrosine Kinase Signaling - an Epithelial Function of the SH2 Domain Protein-Tyrosine Phosphatase SHP-1. *J.Cell Biol.*, **in press**

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